Induction of Suppressor T Cells and Inhibition of Contact Hypersensitivity in Mice by 12-O-Tetradecanoylphorbol-13-Acetate and Its Analogs

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12-O-tetradecanoylphorbol-13-acetate (TPA) and its analogs were surveyed for their abilities to modify contact hypersensitivity (CHS) responses in SENCAR mice. Sensitization of dorsal skin with 2,4-dinitrofluorobenzene (DNFB) and subsequent challenge of the ear 5 d later resulted within 24 h in ear swelling and increased vascular permeability (as measured by the extravasation of Evans Blue dye). Treatment of dorsal or ventral skin with TPA 4 times (application made every 3 or 4 d) prior to sensitization on the dorsum inhibited subsequent induction of CHS by DNFB challenge. Maximum suppression of CHS required sensitization at the site of TPA treatment. Suppression occurred over a narrow dose range of TPA (0.1 – 1.0 μg), and qualitatively correlated with the tumor incidences scored in an initiation-promotion multistage skin carcinogenesis experiment. Multiple applications (4×) of the promoters phorbol-12,13-dibenzoate (10 μg) and mezerein (2 μg) also suppressed CHS, whereas the non-promoter phorbol (20 μg) and the first stage tumor promoter 4-O-methyl TPA (20 μg) had no effect. Adoptive transfer of splenocytes isolated from mice pre-treated with TPA prior to DNFB sensitization inhibited the development of CHS in recipient mice that were sensitized and challenged with DNFB, but not oxazolone. Splenocyte preparations depleted of T lymphocytes prior to transfer could not suppress CHS in recipient mice. Conversely, suppressive activity was concentrated in splenocyte preparations depleted of adherent cells/monocytes. Collectively, these studies demonstrate that TPA treatment of murine epidermis prior to sensitization with hapten can inhibit subsequent hapten-dependent elicitation of CHS. This suppression is mediated in part by antigen-specific suppressor T cells. Furthermore, there is a qualitative correlation between the complete and second stage in vivo tumor-promoting activities of TPA and its analogs, and their abilities to inhibit CHS. *J Invest Dermatol 96:864 – 870, 1991*

Skin tumors develop in SENCAR mice after the topical application of a single, non-tumorigenic dose of carcinogen (initiation) if followed by repetitive treatment of the initiation site with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). The contributions of the immune system to the processes of promotion are unknown; however, co-treatment of mice with immunomodulating agents during the promotion period has been reported to inhibit [1 – 5] or enhance [5,6] tumor multiplicities, and TPA has pronounced effects on both the efferent and afferent components of the immune system. Specifically, cutaneous leukocyte content (e.g., macrophages, neutrophils, mast cells) increase markedly following topical TPA treatment [7 – 9]. Conversely, the morphologies of epidermal Thy-1$^+$ and Langerhans (LC) cells are dramatically altered, and Thy-1$^+$ cell densities are significantly reduced in TPA-treated epidermis [9,10]. As few as two topical applications of TPA are sufficient to double the cellularity of the spleen [11]. This increase represents predominately adherent cells (monocytes) that generate proportionally greater amounts of interleukin 1 and tumor necrosis factor, upon in vitro stimulation with lipopolysaccharide, relative to splenocytes isolated from acetone-treated mice [11]. Splenocytes from TPA-treated SENCAR mice also have reduced natural killer cell activity when assayed in vitro [12,13]. These findings demonstrate that topical treatment of the skin with TPA has both local and systemic effects on components of the immune system. However, it is unclear whether any of these effects result in a functional modulation of the in vivo immune system, or are relevant to the process of TPA-dependent promotion.

Epidermal LC originate in the bone marrow and are involved in the initiation of cutaneous immune responses such as contact hypersensitivity (CHS). Upon exposure of the skin to a sensitizing agent the LC bind, process, and transport the agent to the draining lymph nodes where it is presented to T cells, which facilitates subsequent T-cell – mediated reactions [14,15]. Numerous studies have demonstrated that CHS reactions are dependent upon the relative LC composition of the tissue. Specifically, sensitization through tissues deficient in LC induces immunologic unresponsiveness (tolerance) to subsequent antigen challenge [14,16,17]. Similarly, depletion or alteration of the morphology of LC by exposure to the chemical carcinogen 7,12-dimethylbenz[a]anthracene (DMBA) [18,19] or ultraviolet light [20,21] prior to sensitization induces tolerance to future antigen challenge. This tolerance to subsequent challenge is mediated by antigen-specific suppressor T cells [19,20].

Manuscript received July 11, 1990; accepted for publication December 20, 1990.

This work was supported in part by grants CA 34469 and CA 49935 awarded by the National Cancer Institute, DHHS.

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Abbreviations:

CHS: contact hypersensitivity
DMBA: 7,12-dimethylbenz[a]anthracene
DNFB: 2,4-dinitrofluorobenzene
LC: Langerhans cells
TPA: 12-O-tetradecanoylphorbol-13-acetate

0022-202X/91/$03.50

The current study used the CHS reaction as a tool to determine whether topical application of TPA could modulate an in vivo immune response, and whether this response correlated with the promoting activity of TPA. The precedents for our study are the reports [9,10] that topical application of promoting doses of TPA dramatically alters LC morphology (and possibly their function) in SENCAR mice and the report [22] that topical TPA treatment reduced epidermal LC numbers, and inhibited CHS responses in Balb/c mice. In our study we have used SENCAR mice because tumor responses and epidermal LC densities/morphologies are well characterized in this murine stock with respect to the doses, duration, and scheduling of treatments with TPA, and its promoting and non-promoting analogs. We conclude that there is a qualitative relationship between the promoting activity of TPA and its analogs and their abilities to inhibit CHS responses, and that the suppressive effects of TPA on CHS are mediated in part by antigen specific, suppressor T cells.

MATERIALS AND METHODS

Chemicals 12-O-tetradecanoylphorbol-13-acetate, phorbol dibenzoate, 4-O-methyl TPA, mezerein, and phorbol were purchased from LC Services Corporation (Woburn, MA). 2,4-dinitrofluorobenzene, 4-ethoxymethylene-2-phenyl-oxazolin-5-one (oxazolone), Sephadex G-10, and FITC-conjugated anti-mouse Thy 1.2 were purchased from Sigma (St. Louis, MO). Lympholyte M and Low-Tox-M rabbit complement were obtained from Cedarlane (Westbury, NY). Unlabeled monoclonal anti-murine Thy 1.2 was purified from the supernatant fluids of cell line 30-H12 (American Tissue Culture Collection #TIB 107, Rockville, MD).

Mice Outbred female SENCAR mice (7–9 weeks old, National Cancer Institute, Frederick, MD) were used in all experiments except the adoptive transfer studies. For adoptive transfer studies, inbred female SENCAR mice (SSIN) were obtained from the Science Park–Veterinary Division, Bastrop, Texas.

Sensitization and Elicitation of Contact Sensitivity Dorsal and/or ventral trunks of mice were shaved with surgical clippers one week before treatment. Animals in hair regrowth at the time of initial treatment were not used. Putative modulators of contact sensitivity were dissolved/diluted in acetone and applied topically to the shaved areas in 0.2 ml. Control mice were treated with 0.2 ml acetone. Scheduling and duration of treatments are noted in the Table legends. CHS reactions were performed similar to the protocol reported by Halliday et al [22]. One week after the final pre-treatment, mice were sensitized by applying 25 μl of a 0.5% solution of DNFB or 25 μl of a 5% solution of oxazolone (both made in 4:1 acetone:olive oil) to their dorsal trunks for two consecutive days. For elicitation of contact sensitivity, the inside surface of the right ear was challenged with 20 μl of 0.2% DNFB, or 10 μl of 1% oxazolone (in 4:1 acetone:olive oil) 5 d after the initial sensitization. Solvent alone was applied to the left ear as a vehicle control. Twenty-four hours after challenge, the contact sensitivity response was quantitated by measuring changes in ear thickness and/or vascular permeability. To assess changes in vascular permeability, tail veins were injected (250 μl/30 g body weight) with a 1% solution of Evans blue dye (suspended in PBS) that had been filtered through a 0.22-μm membrane. Mice were anesthetized 30 min later with Ketamine, and ear thickness was measured using an engineer's micrometer (Mitutoyo). The contact sensitivity response was quantitated by the following formula:

$$\frac{\text{thickness of test ear} - \text{thickness of control ear}}{\text{thickness of control ear}} \times 100.$$

Mice were killed by cervical dislocation upon completion of ear measurements. Ears were then removed and a 9-mm diameter punch of the ear was made with a cork borer. Ear punches were transferred to tubes containing 1 ml of extraction buffer (600 μl acetone and 400 μl 0.5% Na_2SO_4) and cut into small pieces inside the tube with scissors. After overnight storage at room temperature the samples were centrifuged and the optical density (OD) of the supernatant fluids was read at 611 nm against a blank consisting of extraction buffer. Changes in vascular permeability are expressed as follows:

$$\frac{OD_{611}\text{ treated ear} - OD_{611}\text{ control ear}}{63.5\text{ mm}^2}.$$

For those studies in which only ear thickness was measured, the intravenous administration of Evans blue dye was deleted.

In initial studies the inflammatory properties of DNFB was examined by treating the ears of non-sensitized mice with DNFB. Changes in ear thickness and vascular permeability were less than 10% of the response obtained in mice that had been previously sensitized with antigen (see Fig 1). Our data have not been corrected for the small contribution of DNFB alone to the inflammatory processes we measured.

Adoptive Transfer of Splenocytes Donor inbred mice were sensitized with DNFB through TPA or acetone-treated dorsal skin as described above. One week after sensitization, spleens were removed from donor mice and placed in Hank's balanced salt solution (HBSS). Single cell suspensions were prepared by passage of the spleens through a stainless steel screen. The resulting cell suspension was pelleted, and then resuspended in 0.155 M NH_4Cl, 16 mM Tris-HCl, pH 7.3, in order to lyse red blood cells. After a 2–3-min incubation at room temperature, the cells were pelleted by centrifugation, and then washed 3 times with HBSS. The washed cell pellet was resuspended in sterile 0.15 M NaCl and injected (0.2–0.3 ml containing 5×10^7 splenocytes) into the tail vein of syngeneic recipient mice. Recipient mice were sensitized on the dorsum with DNFB or oxazolone within 1–2 h of splenocyte transfer, and then again 24 h later. Ears were challenged 5 d after the initial sensitization, and contact-sensitivity responses were assessed as described above.

T-Cell Depletion Splenocyte suspensions were washed 3 times in HBSS before being resuspended in RPMI 1640 medium containing heat-inactivated 10% fetal bovine serum (FBS) and anti-Thy 1.2. After 1 h incubation at 4°C the cells were washed twice with RPMI 1640 before being resuspended in RPMI 1640 containing 10% FBS and Low-Tox-M rabbit complement. After 1 h incubation at 37°C the splenocytes were washed 2 times with HBSS and viable cells were separated from red blood cells and dead cells on lympholyte M gradients, according to the manufacture's instructions. Viable cell fractions were pooled, washed 3 times in HBSS and processed for injection as described above. As a control, a portion of the unfractionated suspension was carried through a parallel procedure that excluded exposure of the splenocytes to anti-Thy 1.2. T-cell depletion was monitored by FACS analyses of splenocyte binding of FITC-conjugated anti-Thy 1.2.

Adherent Cell Depletion Monocytes and macrophages were removed from splenocyte suspensions by the procedure of Ly and Mishell [23]. Splenocytes were suspended in RPMI 1640 containing 10% FBS and loaded (3 ml with 8×10^7 cells/ml) onto G-10 Sephadex columns (bed volume of 10 ml) that had been washed with HBSS containing 5% FBS. The column was washed with RPMI 1640 containing 5% FBS and the non-adherent cells (T cells) eluted in the first 30 ml. After being washed 4 times in HBSS, the cells were processed for injection as described above.

Statistical Analyses Differences between control and treatment groups were evaluated by the Student t test or by analyses of variance (ANOVA) using the Fisher protected least significant difference (PLSD) test. Significance was set at $p < 0.05$. Each experimental group contained 5–8 animals, and each experiment was repeated at least twice.

RESULTS

Kinetics of CHS Response Contact-sensitivity reactions require prior exposure (sensitization) of the animal to antigen. Subse-

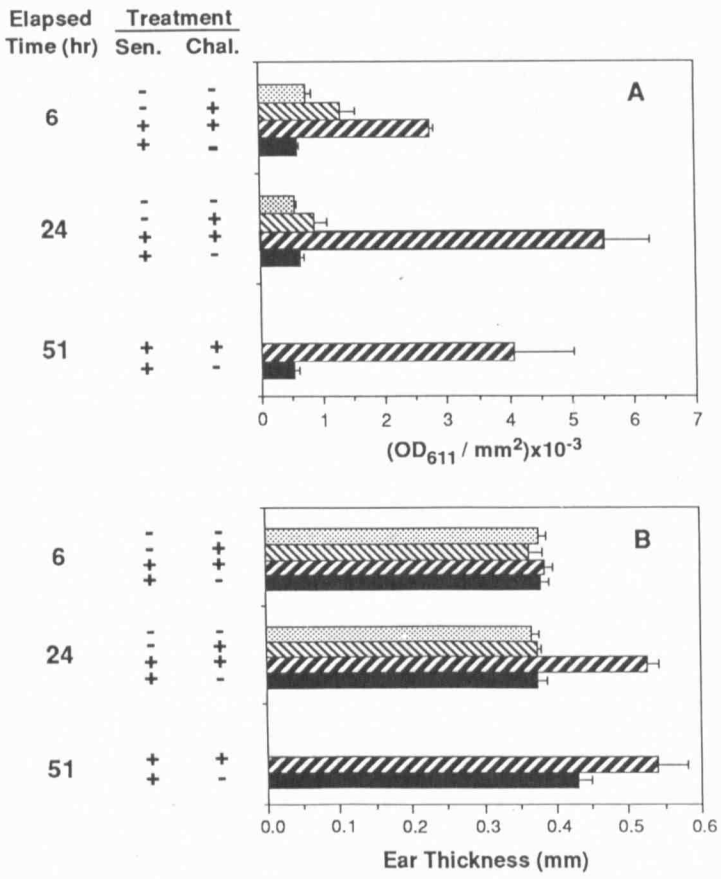

Figure 1. Kinetics of changes in vascular permeability *(A)* and ear swelling *(B)* following elicitation of contact hypersensitivity. The dorsal skins of SENCAR mice were treated 4 times (2×/week for 2 weeks) with acetone prior to sensitization with DNFB, or treatment with solvent vehicle. Mice were sacrificed 6, 24, and 51 h after DNFB challenge or solvent vehicle treatment of ears.

quent treatment (challenge) of previously sensitized animals on the ear with antigen leads to erythema, edema, and swelling of the ear. The kinetics of changes in ear thickness and vascular permeability following antigen challenge of sensitized mice are shown in Fig 1. Changes in vascular permeability were noted within 6 h of challenge, and peaked ~18 h later (Fig 1*A*). Thereafter, there was a decline in permeability. Changes in ear thickness were obvious within 24 h of challenge, and were sustained for an additional 27 h (Fig 1*B*). Treatment of the ears of non-sensitized mice with only DNFB caused no ear swelling over the period of observation, and only a minor increase in permeability within 6 h of topical application. This DNFB-induced change in vascular permeability measured in non-sensitized mice declined to background levels by 24 h post-treatment. In subsequent studies, CHS responses were measured ~24 h after antigen challenge.

The study depicted in Fig 1 employed mice that were sensitized on dorsal skins that had been previously treated 4 times with acetone. Multiple solvent treatments were used to better approximate the multiple chronic treatments used in a tumor promotion protocol.

Inhibition of CHS by TPA A single treatment of SENCAR dorsal skin with 2 μg of TPA prior to sensitization with 0.5% DNFB strongly inhibited subsequent induction of CHS in ears challenged with DNFB (~92% suppression, Table I). Similarly, multiple pretreatments with TPA (2 μg, 2 times/week for 2 weeks) inhibited the swelling of ears challenged with DNFB (~96% suppression). Although it appears in this experiment that multiple

Table I. Effects of Multiple TPA Treatments on CHS[a]

Applications	Percent Increase in Ear Thickness	
	Acetone	TPA
1	21.3 ± 2.6	1.6 ± 0.8[b]
4	50.3 ± 6.1	1.2 ± 0.3[b]

[a] The dorsal skins of SENCAR mice were treated either once or 4 times (2×/week for 2 weeks) with either acetone or 2 μg of TPA prior to sensitization with a 0.5% solution of DNFB. Treatments were coordinated such that all mice were sensitized, challenged, and sacrificed on the same days.

[b] Significantly less than acetone controls, $p < 0.001$.

treatments of the dorsum with acetone prior to sensitization potentiates ear swelling, we have not consistently noted this potentiation in other experiments.

TPA-dependent suppression of CHS occurred over a very limited dose range (Table II). Multiple applications of 0.1 μg TPA had no statistically significant effects on CHS; whereas, treatment with 1.0 μg resulted in significant, and maximum suppression.

The degree of suppression of CHS afforded by the various doses of TPA paralleled papilloma incidence obtained in an in vivo initiation-promotion study (Table II). Few animals developed papillomas following multiple treatments with 0.1 μg TPA, whereas tumor incidence was 100% in mice treated repeatedly with 1 μg TPA.

Modulation of CHS Responses by Analogs of TPA Phorbol dibenzoate (PDB), at a dose of 10 μg, is a weak tumor promoter when applied chronically to initiated CD-1 mice, a strain from which the SENCAR was derived [24]. Treatment of dorsal skin with PDB prior to sensitization with DNFB inhibited CHS-mediated ear swelling. However, the magnitude of the suppression varied considerably between experiments (Table III). Pre-treatment with a non-hyperplastic, non-inflammatory, and non-promoting dose (20 μg) of phorbol [24] did not inhibit CHS-mediated ear swelling. Similarly, pretreatment with 4-O-MeTPA, at a dose (20 μg) characterized as being a first-stage promoter [25], had no effect on CHS-associated ear swelling (Table III). Mezerein, which is a modest complete promoter [26] but a potent second-stage promoter [25] in the murine skin multistage carcinogenesis protocol, was as effective as TPA, on the basis of dose, in suppressing CHS-mediated ear swelling (Table III).

Adoptive Transfer of Splenocytes An inbred derivative of SENCAR mice (designated SSIN) has been described that is more sensitive than the outbred stock to TPA-dependent promotion [27]. Like the outbred SENCAR, CHS responses were significantly suppressed in SSIN mice when dorsal skin was treated with TPA prior to sensitization with DNFB (control groups in Tables IV and V). The suppressive effects of TPA could be transferred between mice

Table II. Dose-Dependent Inhibition of CHS by TPA[a]

Treatment	Dose (μg)	Percent Increase in Ear Thickness		Papilloma Incidence[b]
		Experiment 1	Experiment 2	
Acetone		37.6 ± 3.9	25.8 ± 2.2	0
TPA	0.1	28.1 ± 4.0	28.5 ± 1.3	6
TPA	0.25	[c]	[c]	15
TPA	0.5	16.2 ± 6.2[d]	20.3 ± 3.3	70
TPA	1.0	11.3 ± 2.5[d]	3.1 ± 0.9[d]	100
TPA	2.0	15.8 ± 2.7[d]	4.5 ± 1.2[d]	100

[a] The dorsal skins of SENCAR mice were treated 4 times (2×/week for 2 weeks) with either acetone or various doses of TPA prior to being sensitized with a 0.5% solution of DNFB.

[b] Percentage of SENCAR mice initiated with DMBA and promoted with acetone or varying doses of TPA 2×/week for at least 30 weeks that developed papillomas. Data are from [40] and author's unpublished studies.

[c] Not performed.

[d] Significantly less than acetone controls, $p < 0.05$.

Table III. Modulation of CHS by Analogs of TPA[a]

Treatment	Dose (μg)	Percent Increase in Ear Thickness		
		Experiment 1	Experiment 2	Experiment 3
Acetone		34.9 ± 7.2	25.8 ± 2.3	54.1 ± 4.5
Phorbol	20	31.7 ± 3.8		
4-O-MeTPA	20	27.9 ± 4.6	28.1 ± 0.7	
PDB	10	23.3 ± 4.9		16.0 ± 3.6[b]
Mezerein	2	13.8 ± 2.7[b]	5.1 ± 1.2[b]	
TPA	2	16.3 ± 3.2[b]		13.1 ± 3.4[b]
TPA	1		3.1 ± 0.9[b]	

[a] The dorsal skins of SENCAR mice were treated 4 times (2×/week for 2 weeks) with either acetone or various chemicals prior to being sensitized with a 0.5% solution of DNFB.
[b] Significantly less than acetone treated control, $p < 0.05$.

by the adoptive passage of splenocytes (donor groups, lines 3 and 4 of Tables IV and V). Specifically, CHS-mediated ear swelling following DNFB challenge was markedly inhibited in mice which received, just prior to DNFB sensitization, preparations of splenocytes from donor mice that had been pre-treated with TPA prior to DNFB sensitization.

Two approaches were used to characterize the splenocyte cell type that was responsible for inhibition of CHS responses. First, columns of G-10 Sephadex were used to remove a majority of the adherent cells in the splenocyte suspensions (Table IV). Ear swelling and changes in vascular permeability were strongly suppressed following DNFB challenge in mice receiving splenocyte populations that had been depleted of monocytes. Conversely, splenocyte populations depleted of their T cells (by lysing with anti-Thy 1.2 plus complement) were totally unable to suppress CHS-mediated increases in ear swelling and vascular permeability. Treatment of splenocytes with only complement had no effect on the suppressive activities of the splenocyte suspensions. Collectively, these studies suggest that it is a T-cell component of the splenocyte cell suspension that is responsible for mediating the TPA-dependent suppression of CHS reactions.

The suppressive activity of the adoptively transferred splenocyte suspensions was antigen specific (Table V). Specifically, splenocytes prepared from donor mice that had been pre-treated with TPA prior to sensitization with DNFB could inhibit CHS responses in recipient mice that were sensitized and challenged with DNFB. However, the same preparation of splenocytes, when transferred just prior to sensitization of recipient mice with oxazolone, could not inhibit CHS-mediated ear swelling that occurred following subsequent challenge with oxazolone. This antigen specificity further suggests that the TPA-dependent modulation of CHS is mediated by suppressor T cells.

Systemic Modulation of CHS by TPA Maximum TPA-dependent suppression of CHS required sensitization through skin that had been previously treated with TPA (Table VI). However, if TPA was applied to the ventral surface of mice, and mice were sensitized on the dorsal skin (previously treated with acetone), there was some suppression of CHS-mediated ear swelling and changes in vascular permeability following DNFB challenge on the ears. The study reported in Table VI has been performed 4 times, and in three studies the ventral application of TPA significantly suppressed CHS (~40–60% suppression, $p < 0.05$). In these studies, the levels of suppression obtaining by sensitizing at a site distant from the site of TPA application were approximately 53 to 85% of the values obtained when sensitization occurred at the site of TPA treatment.

Correlation Between Ear Swelling and Vascular Permeability Although CHS reactions are commonly monitored by measuring ear thickness, the assay is somewhat subjective and dependent upon the skill and experience of the investigator. Consequently, it would be helpful to have an alternative assay that could be performed on the same tissue.

The ear swelling that occurred following antigen challenge reflected changes in the vascular permeability of the ear (Fig 2). In Fig 2 we set control CHS responses as 100% and plotted the effects of modulators of CHS on ear thickness versus vascular permeability. Ear swelling quantitatively correlated with the extravasation of Evans Blue dye in the tissue ($r = 0.68$ for the least-squares fit of the line). The data in Fig 2 strongly suggest that suppressions in ear thickness of ≥50% should be corroborated by decreases in vascular permeability of ≥35%.

DISCUSSION

Two groups have reported that application of TPA prior to, and at the site of, treatment with a contact-sensitizing agent inhibits subsequent induction of CHS [22,28]. In both studies a single dose of TPA was employed. The current study demonstrates a close similarity between the dose-response curves describing the TPA-dependent suppression of CHS and the in vivo promoting activity of TPA, as scored as tumor incidence in the murine skin multistage carcinogenesis model. Analogs of TPA having in vivo promoting activity also suppressed CHS, whereas, non-promoting analogs were not suppressive. In addition, the analog studies suggest that the CHS suppressive activity of TPA may be associated with the operationally defined second stage of tumor promotion in mouse skin. Al-

Table IV. Inhibition of CHS by Adoptive Transfer of Splenocytes and T cells[a]

Mouse Group	Treatment	Splenocyte Treatment	Percent Increase in Ear Thickness	(OD_{611}/mm^2) × 10^{-4}
Control	Acetone		39 ± 9 (100)[c]	89 ± 7 (100)
Control	TPA		25 ± 6 (64)	53 ± 8[b] (59)
Donor	Acetone	None	46 ± 7 (100)	96 ± 15 (100)
Donor	TPA	None	17 ± 6[b] (37)	59 ± 12[b] (61)
Donor	TPA	G-10 filtered	7 ± 4[b] (15)	33 ± 8[b] (34)
Donor	TPA	Anti-Thy 1.2 + complement	54 ± 12 (117)	102 ± 18 (106)
Donor	TPA	Complement	12 ± 3[b] (26)	44 ± 4[b] (46)

[a] The dorsal skins of control and donor mice were treated 4 times (2×/week for 2 weeks) with either acetone or 1 μg of TPA prior to being sensitized with 0.5% DNFB. Control mice were challenged 5 d later. Splenocytes of donor mice were prepared 7 d after the first sensitization of donors and transferred to recipient mice, or first passed through Sephadex G-10 columns to remove adherent cells (monocytes and macrophages), or treated with anti-Thy 1.2 plus complement to destroy T cells, before being transferred to recipient mice. Recipient mice were sensitized with DNFB within 2 h of receiving the splenocytes (5×10^7 cells). Treatments were coordinated such that control and recipient mice were sensitized, challenged and sacrificed at the same time.
[b] Significantly different than corresponding acetone treated control or donor mice, $p < 0.05$.
[c] Values in parentheses are percentages of control or donor acetone values.

Table V. TPA-Dependent Suppression of CHS by Adoptively Transferred Splenocytes is Antigen Specific[a]

Group	Treatment	Agent: Sensitization of Control or Donor	Agent: Sensitization of Recipient	Challenge	Percent Increase in Ear Thickness
Control	Acetone	DNFB		DNFB	74 ± 1 (100)[c]
Control	TPA	DNFB		DNFB	25 ± 6[b] (34)
Donor	Acetone	DNFB	DNFB	DNFB	48 ± 7 (100)
Donor	TPA	DNFB	DNFB	DNFB	23 ± 7[b] (48)
Donor	Acetone	DNFB	Oxazolone	Oxazolone	122 ± 24 (100)
Donor	TPA	DNFB	Oxazolone	Oxazolone	104 ± 10 (85)

[a] The dorsal skins of SENCAR mice were treated 4 times (2×/week for 2 weeks) with either acetone or 1 μg of TPA prior to being sensitized with 0.5% DNFB. Control mice were challenged 7 d after the first sensitization. Splenocytes of donor mice were prepared and transferred to recipient mice 7 d after the first sensitization with DNFB. Recipient mice were sensitized with either DNFB or oxazolone within 2 h of receiving the splenocytes (5×10^7 cells). Treatments were coordinated such that control and recipient mice were sensitized, challenged and sacrificed at the same time.

[b] Significantly different than corresponding acetone treated control or donor mice, $p < 0.05$.

[c] Values in parentheses are percentages of control or donor acetone values.

though suppression of CHS by TPA and its analogs appears to correlate with the promoting activities of the chemicals, it should be emphasized that these data do not address whether the immunosuppressive/immunomodulating properties of TPA are relevant to the processes of tumor promotion.

Halliday et al [22] previously reported that the TPA-dependent suppression of DNFB-induced CHS could be passaged between mice by the intravenous adaptive transfer of splenocytes. In the current study we demonstrate that it is the T-cell component of the spleen, and not the adherent cell population, that mediates this suppression. This clarification is significant because TPA treatment of SENCAR mice increases the splenic content of monocytes [11], and these cells are primed and have the capacity to produce increased amounts of IL-1 [11], which has been reported to be an inhibitor of CHS [29]. Furthermore, we have been able to show that the suppressive effects of adoptively transferred splenocytes is antigen specific. Collectively, these findings suggest that the TPA-dependent suppression of CHS measured in the current study is mediated in part by suppressor T cells.

Maximum suppression of CHS by TPA required sensitization at the site of TPA treatment. Although quantitatively less, significant suppression also occurred when the sites of sensitization and TPA application were distant from one another. This suggests that there are both local and systemic effects of TPA on CHS. With regard to the local effects, numerous studies have demonstrated that sensitization through skin either naturally deficient in LC cells [14,16,17] or made deficient by UV [20,21] or carcinogen [18,19] treatments results in the generation of suppressor T cells. Given the reported effects of TPA on epidermal LC densities [22] and morphologies [9,10] in Balb/c and SENCAR mice, respectively, and the role of LC in antigen presentation [14–17], the suppressor T-cell population generated following DNFB sensitization through TPA-treated skin may reflect promoter-dependent alterations in antigen processing/presentation. This explanation cannot be evoked to explain the suppressive effects of TPA painted at a site distant from sensitization. Several studies have demonstrated that in vitro treatment of bovine and human leukocytes with TPA results in changes in T-cell marker expression and function that suggest a TPA-dependent induction of suppressor cells [30–32]. In one of these studies [30] there was a direct correlation between the in vivo promoting activities of TPA and its analogs, and their abilities to induce the maturation of a T-cell subpopulation into suppressor T cells. Although in vitro studies have demonstrated that epidermal homogenates can hydrolyze TPA to non-promoting metabolites [33], topically applied TPA is poorly metabolized in vivo by the skin, and rapidly cleared from the skin [33]. Furthermore, although esterase 1 in mouse plasma [34] is capable of hydrolyzing TPA to inactive metabolites, as are esterases in the liver [35], sufficient amounts of topically applied TPA have been reported to reach internal organs [36,37] capable of evoking responses that can be mediated by TPA but not its metabolites. Consequently, we cannot rule out a systemic effect of topically applied TPA on the maturation pathway of T cells. Similarly, we cannot rule out the possibility that topically

Table VI. Effects of TPA on CHS when Applied at a Site Different than that Used for Sensitization[a]

Treatment	Site of Treatment	Site of Sensitization	Percent Increase in Ear Thickness
Acetone	Dorsal	Dorsal	42.7 ± 2.0
TPA	Dorsal	Dorsal	9.6 ± 2.9[b]
TPA	Ventral	Dorsal	25.1 ± 4.2[b]

[a] The dorsal or ventral skins of SENCAR mice were treated 4 times (2×/week for 2 weeks) with either acetone of 2 μg of TPA prior to sensitization with a 0.5% solution of DNFB.

[b] Significantly different from acetone controls, $p < 0.05$.

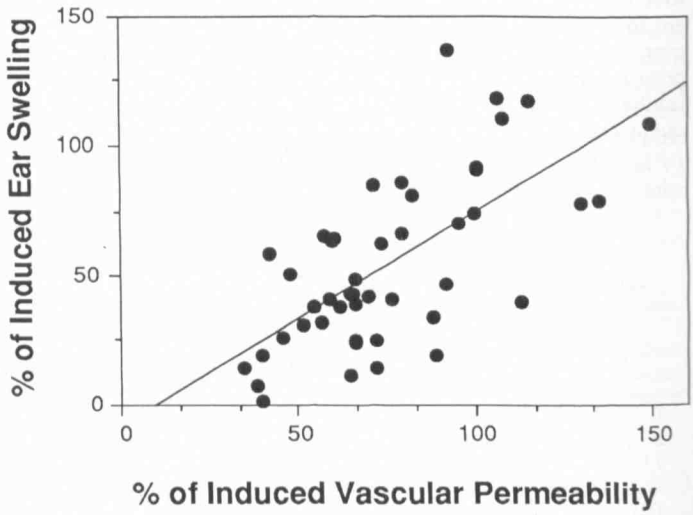

Figure 2. Relationship between changes in ear swelling and vascular permeability following the induction of CHS. The dorsal skins of mice were treated 4 times (2×/week for 2 weeks) with acetone, or TPA, or its analogs prior to sensitization with DNFB. Vascular permeability and ear thickness measurements made in acetone-treated mice were set as 100%. *Point,* the mean of a single 5 to 8 mouse experimental group versus the mean of its corresponding 5 to 8 mouse acetone control group. *Line,* least-squares fit of the data with $r = 0.68$.

applied TPA stimulates the production of factors by the skin that contribute to the systemic modulation of CHS and the generation of suppressor cells. Specifically, topical treatment of mice with TPA results in IL-1 and PGE_2 production by keratinocytes [38,39]. Both IL-1 and PGE_2 have been implicated as modulators of CHS reactions in mice [29].

There are some striking similarities between the immunosuppressive effects of UV irradiation and topically applied TPA. Specifically, exposure of mice to UV either at the site of, or distant from the site of contact sensitization, results in immune tolerance to subsequent challenge [20,21]. Like the results obtained with TPA, the UV-dependent suppression of CHS is mediated by antigen-specific suppressor T cells [20,21]. Whether these results are simply coincidental or suggestive of a common mechanism of immunosuppression shared by TPA and UV will only be answered by further studies.

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FASEB SUMMER RESEARCH CONFERENCES

The Federation of American Societies for Experimental Biology will again present its summer research conference series. The conferences are designed to address current topics in biology and medicine that are of intense scientific interest. Participants are encouraged to discuss and exchange information on the cutting edge of biomedical research while enjoying the quiet and unique settings of these conferences. Each conference meets for a five day period at one of the selected sites: Saxtons River, Vermont or Copper Mountain, Colorado. The attendance is limited to 150 scientists and will be by invitation upon application. Further information and an application are available in the February issue of the *FASEB Journal* or can be obtained by contacting the Conference Office, (301) 530-7093.

1991 SCHEDULE AND ORGANIZERS

Saxtons River, Vermont

Ubiquitin and Protein Degradation, June 9–14: Alfred Goldberg, Harvard Medical School, *Chair;* Richard Vierstra, University of Wisconsin, *Vice-Chair.*

Neuroimmunology, June 16–21: Edward G. Goetzl, University of California/San Francisco, *Chair;* Jean Merrill, University of California, Los Angeles, *Vice-Chair.*

Lymphocytes and Antibodies, June 23–28: David Parker, University of Massachusetts Medical School, *Chair;* Laurie Glimcher, Harvard School of Public Health, *Vice-Chair.*

Transgenic Animals: Current Status and Future Prospects, June 30–July 5: Jon W. Gordon, The Mount Sinai Medical Center/New York, *Chair;* George A. Scangos, Bayer AG/West Germany, *Vice-Chair.*

Cellular and Molecular Genetics, July 7–12: Kathryn Calame, Columbia University College of Physicians and Surgeons, *Chair;* David Livingston, Harvard Medical School, *Vice-Chair.*

Positive Control of Transcription Initiation of Prokaryotes, July 14–19: Sankar Adhya, National Cancer Institute/NIH, *Chair;* Susan Garges, National Cancer Institute/NIH, *Vice-Chair.*

Molecular Mechanisms of Carcinogenesis, July 21–26: Michael Lieberman, Baylor College of Medicine, *Chair;* Peter Howley, National Cancer Institute/NIH, *Vice-Chair.*

Genetic Recombination and Genome Rearrangements, July 28–August 2: Richard Kolodner, Dana-Farber Cancer Institute, *Chair;* Rodney Rothstein, Columbia University College of Physicians and Surgeons, *Vice-Chair.*

Low Molecular Weight GTP Binding Proteins, August 4–9: Gary Bokoch, Research Institute of Scripps Clinic, *Chair;* Channing J. Der, La Jolla Cancer Research Foundation, *Vice-Chair.*

Modulation of Wound Healing, August 11–16: Thomas Hunt, University of California/San Francisco, *Chair;* William Lindblad, Wayne State University, *Vice-Chair;* H. Paul Ehrlich, Shrine Burn Unit of Massachusetts, *Vice-Chair.*

Copper Mountain, Colorado

Chromatin and Transcription, June 23–28: Gordon Hager, National Cancer Institute/NIH, *Chair;* Michael Grunstein, Molecular Biology Institute, UCLA, *Vice-Chair.*

Neurotransmitters and Modulators in Opioid Analgesia, June 30–July 5: Thomas Burks, University of Texas Health Science Center, Houston, *Chair;* Brian Cox, Uniformed Services University of Health Sciences, *Vice-Chair.*

Protein Kinases, July 7–12: Jackie Corbin, Vanderbilt University School of Medicine, *Chair;* Michael Czech, University of Massachusetts Medical Center, *Vice-Chair.*

Smooth Muscle, July 14–19: James T. Stull, Southwestern Medical Center, *Chair;* Cornelius van Breemen, University of Miami, *Vice-Chair.*

Biology and Chemistry of Vision, July 21–26: Bernard Fung, University of California School of Medicine/Los Angeles, *Chair;* John Lisman, Brandeis University, *Vice-Chair.*

Endothelium and Cardiovascular Function, July 28–August 2: Paul M. Vanhoutte, Baylor College of Medicine, *Chair;* Rudi Busse, Institute of Applied Physiology, FRG, *Vice-Chair;* R. Wayne Alexander, Emory University, *Vice-Chair.*

Gastrointestinal Tract IV: Development and Repair — Cellular and Molecular Aspects, August 4–9: Michael Gershon, Columbia University College of Physicians & Surgeons, *Chair;* Marion Neutra, Harvard University, *Vice-Chair.*

Cytokines and Lipid Mediators as Regulators of Cell Function, August 11–16: Patrick Y-K. Wong, New York Medical College, *Chair;* Robert Lewis, Syntex Corporation, *Vice-Chair.*